Synthesis of 4-Sulfamoylapigenin as a Potential Antiproliferative Agent

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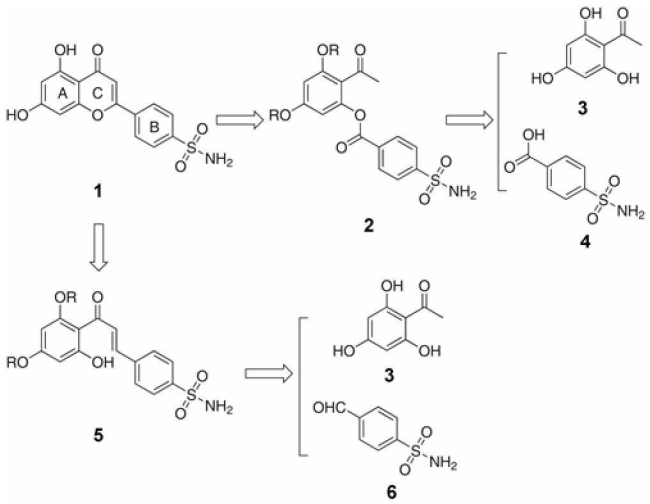
It is widely accepted that inhibition of cyclin-dependent kinases (CDKs) could provide control of the inappropriate cellular proliferation characteristic of certain cancers, and compounds able to inhibit the activity of these crucial enzymes are expected to have antiproliferative effects. Due to the overall success of flavopiridol,[1] and the availability of the X-ray structure of *des*-chloroflavopiridol co-crystallized with CDK2,[2] numerous flavonoid analogues have been prepared and their biological activities have been estimated.[3] Unfortunately, however, in spite of studies on a wide range of flavonoid compounds, no clear structure-activity relationships (SAR) regarding their antiproliferative effects could be drawn.[3(f)] In this study, we reasoned that presumably, at least in part, the lack of the SAR results from the low solubility of the flavonoids, which affects the effective concentration through precipitation in aqueous media.[4] Based on this assumption, we attempted to install hydrophilic polar functionalities around the flavone scaffold. As it is clear from the X-ray structure of *des*-chloroflavopiridol,[2] ring A of flavone is located inside the cleft of the ATP-binding site whereas ring B is exposed outside. Thus, ring B would be the place of choice for installation of the polar functionality. To our knowledge, ring B substitution has rarely been explored, and the study of benzofuranone mimics of flavopiridol performed by Schoepfer *et al.*[5] is the only literature precedent dealing with several polar para-substituents on the phenyl ring of 2-benzylidene-benzofuran-3-one, which corresponds to the ring B of flavonoids. Among several candidate polar functional groups, a *p*-sulfonamide substituent was envisaged to reinforce binding to CDK2 through formation of hydrogen bonds with amino acid backbones working both as a hydrogen bond donor (amino group of the sulfonamide) and an acceptor (oxygen atoms of the sulfonamide).

Taken together, 4'-sulfamoylapigenin (**1**, Scheme 1) was chosen as the title compound, which was expected to be more water-soluble and possess potent antitumor activity by enhanced binding affinity to the target enzyme, CDK2. In this synthesis, we originally envisioned that 2-acetyl-3,5-dihydroxyphenyl 4-sulfamoylbenzoate **2** would serve as a key intermediate which can be easily prepared from the condensation of the 2,4,6-trihydroxyacetophenone **3** and *p*-sulfamoylbenzoic acid **4** (Scheme 1). The Baker-Venkataraman rearrangement[6] followed by acid catalyzed cyclization of **2** would, then, provide the target compound **1**. Alternatively,



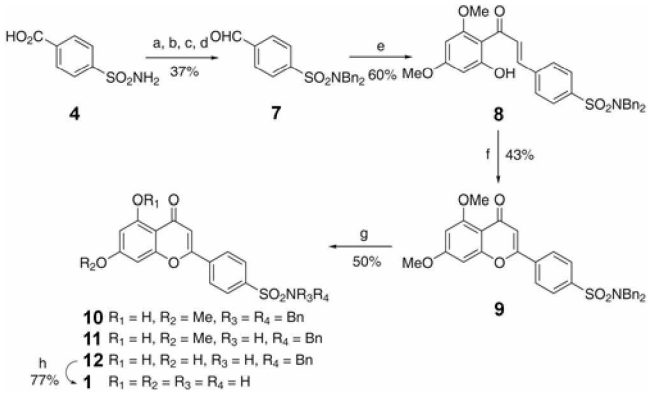
Scheme 1. Retrosynthetic analysis of the title compound (**1**).

aldol condensation of aromatic aldehyde **6** and 2,4,6-trihydroxyacetophenone **3** would provide 4-[3-oxo-3-(2,4,6-trihydroxy-phenyl)-propenyl]-benzenesulfonamide **5** which, upon oxidative cyclization, would construct the flavone skeleton **1**.

At first, we set out the synthesis by adapting the Baker-Venkataraman rearrangement[6] of **2** which is easily accessible from the commercially available starting materials. However, compound **2** did not give the cyclized product **1** upon treatment with K_2CO_3 in refluxing pyridine.[6] Also, protection of the sulfamoyl group of **2** with dimethyl formamidine[8] followed by cyclization was attempted, but the desired apigenin analogue was not obtained.

At this point, we reasoned that the general failure of the Baker-Venkataraman rearrangement[6] followed by acid catalyzed cyclization encountered in these syntheses might result from the instability of the trialkoxyacetophenone aryl ester **2** conferred by the presence of the strong electron withdrawing *p*-sulfonamide group. As cyclization conditions require strongly acidic or basic conditions, these unstable compounds seem to hydrolyze during the cyclization reaction.

Other than the Baker-Venkataraman method, chalcone formation followed by oxidative cyclization has been widely used as an alternative for this purpose.[7] Thus, a chalcone analogue **8** was prepared by aldol condensation of 4,6-dimethoxy-2-hydroxyacetophenone with 4-(*N,N*-bisbenzyl-sulfamoyl) benzaldehyde **7** which was prepared from **4** by



Scheme 2. Synthesis of the title compound **1** by oxidative cyclization of chalcone **9**.

way of successive esterification, benzyl protection,[9] LAH-reduction, and oxidation (Scheme 2). For successful aldol condensation, the purity of aldehyde **7** was critical. Due to the low solubility of the desired product chalcone **8**, purification was performed by repeated recrystallization with dichloromethane. Several oxidative cyclization conditions of the chalcone **8** using I_2 or other oxidants (SeO_2, DMSO, *tert*-BuOH, reflux;[10] $InBr_3$, silica gel, reflux[11]) were tested but the reaction did not proceed at all leaving the starting chalcone unchanged. The unsusceptibility of the chalcone **8** to the reaction conditions is believed to stem from its low solubility and, thus, use of I_2 under refluxing pyridine[12] smoothly converted **8** into the desired product **9** in 43% yield. Global deprotection of methyl ether as well as benzyl amino groups of **9** was attempted by treatment with BBr_3 in dichloromethane at 0 °C. It is worth to note that a series of deprotected products (**10-12**) were observed depending upon the reaction time, and that deprotection occurred in the order of 5-OMe > 4-SO_2NBn_2 > 7-OMe. After stirring the reaction mixture for 6 days at 0 °C, however, a mono-benzylated product **12** was obtained as the sole product in 50% yield. Final deprotection of the benzyl amino group (4-SO_2NHBn) was accomplished by treatment of the mono-benzyl product **12** in acidic conditions to give the title compound **1**[13] in 77% yield.

In summary, even though electron withdrawing character of the 4-sulfamoyl group at the C ring as well as low solubility of the intermediates hampered the synthesis, we completed the first synthesis of flavonoid with polar and electron withdrawing 4-substituent by using oxidative cyclization of the chalcone **8** as the key step. Preliminary cytotoxicity assay of the title compound **1** was performed on Huh7 cells, which showed weak and delayed cytotoxicity; at 100 μM concentration of **1**, 20% and 40% of cell death was observed after 48 hour and 100 hour incubation, respectively. Further studies on the mechanism of this compound as well as SAR study with various A ring substituents are in progress, which will be reported in due course.

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13. Spectroscopic data for compound **1**: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 8.14 (d, J = 8.7 Hz, 2H), 7.90 (d, J = 8.7 Hz, 2H), 6.77 (s, 1H), 6.48 (s, 1H), 6.18 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 183.4, 165.9, 163.8, 163.4, 159.3, 148.1, 135.9, 128.3, 128.0, 108.0, 106.0, 100.5, 95.4; HRMS (ESI): (M + Na) calcd. for $C_{15}H_{11}NO_6SNa$, 356.0205; found, 356.0211.